At page 88, lines 4-5, replace "Horsch, et al., *Nature* 227:1229-1231 (1985))" with --Horsch, et al., *Science* 227:1229-1231 (1985))--

A marked-up version of specification pages 19, 84 and 88 showing the amendments made is attached. 37 C.F.R. §1.121(c)(1)(ii).

REMARKS

Applicants have amended the Application to correct the prior art references cited on page 19, 84 and 88. No new matter has been added by virtue of the amendments made to the specification.

INFORMATION DISCLOSURE STATEMENT

In accordance with the provisions of 37 C.F.R. §1.56, §1.97 and §1.98, Applicants wish to bring the following references, References AA-AB, BA-BZ, CA-CZ, DA-DR, DT-DZ and EA-EL cited on the attached PTO-1449 Form to the attention of the Examiner.

It is respectfully submitted that each of the documents shown on PTO-1449 be made of record in this Application.

Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

Date: 11/08/01

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CERTIFICATE OF MAILING

I hereby certify that this PRELIMINARY AMENDMENT AND INFORMATION DISCLOSURE STATEMENT, PTO-1449 FORM (8 pages) and REFERENCES AA-AB, BA-BZ, CA-CZ, DA-DR, DT-DZ and EA-EL being deposited with the United States Postal Service as first class mail in an envelope addressed to: ASSISTANT COMMISSIONER FOR PATENTS, Washington, D.C. 20231 on this 8th day of November, 2001.

Melissa A. Jackson

Natl. Acad. Sci. USA 96:388-393 (1999); Yamazaki, et al., *J. Am. Chem. Soc.*, 120:5591-5592 (1998)).

Protein splicing *in trans* has recently been described both *in vivo* and *in vitro* (Shingledecker, et al., *Gene* 207:187 (1998), Southworth, et al., *EMBO J.* 17:918 (1998); Mills, et al., *Proc. Natl. Acad. Sci. USA*, 95:3543-3548 (1998); Lew, et al., *J. Biol. Chem.*, 273:15887-15890 (1998); Wu, et al., *Biochim. Biophys. Acta* [35732:1 (1998b)] 1387:422-432 (1998b), Yamazaki, et al., *J. Am. Chem. Soc.* 120:5591 (1998), Evans, et al., *J. Biol. Chem.* 275:9091 (2000); Otomo, et al., *Biochemistry* 38:16040-16044 (1999); Otomo, et al., *J. Biolmol. NMR* 14:105-114 (1999); Scott, et al., *Proc. Natl. Acad. Sci. USA* 96:13638-13643 (1999)) and provides the opportunity to express a protein as two inactive fragments that subsequently can undergo ligation to form a functional product (Figure 2).

Trans-protein splicing also occurs naturally in *Synechocystis* sp PCC6803 (Wu, H., et al., *Proc. Natl. Acad. Sci.* 95:9226 (1998)), where it is essential for forming a functional DNA polymerase III by joining two fragments of the DnaE protein, encoded by two genes separated by 750 kb of chromosomal DNA (Figure 3).

These observations led the present inventors to investigate whether a functional gene product could be generated by splitting the gene of interest into two fragments

the control of pTac promoter and confers resistance to ampicillin.

p215EN2 or p235EN2 were constructed by ligating the *Nco*I to *Kpn*I fragment of pCE215DnaE or pCE235DnaE into the same sites of pCEN2. p215EN2 or p235EN2 has the N-terminus of EPSPS (residues 1-215 for p215EN2, 1-235 for p235) fused to the IN_n.

The *Nco*I to *Fsp*I fragment of pCYB3 was ligated into the *Nco*I to *Dra*I sites of pKEB1 to generate pKEB12 (NEB#1282). A sample of pKEB12 plasmid transformed in *E. coli* strain ER2566 has been deposited under the terms and conditions of the Budapest Treaty with the American Type Culture Collection on May _____, 2000 and received ATCC Patent Accession No. _____. This vector has the C-terminal 36 amino acid residues of the *Ssp* DnaE intein (IN_n) fused to CBD and confers resistance to kanamycin.

pEPS#28 and pEPS#29 were constructed by ligating the *Bgl*II to *Pst*I fragment of pCE215DnaE and pCE235DnaE into the same sites of pKEB12. pEPS#28 or pEPS#29 has the C-terminus of EPSPS (residues 216-427 for pEPS#28, 236-427 for pEPS#29) replacing the CBD in pKEB12 and attached to the C-terminus of IN_C.

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through. The plant promoters were functional upon transformation in to *E.coli* and trans-spliced products (aadA-smGFP fusion protein, 57 kDa) were observed in Western blot assay using anti GFP antibodies. Thus chloroplast specific promoters are functional in *E.coli* and could be used for gene expression studies.

The following protocol describes the production of a *E.coli*/plant shuttle vector (pNCT114/pNCT224) that is capable of homologous recombination of a transgene(s) *in vivo*.

A shuttle vector consists of elements that will make it functional in both *E.coli* as well as plant cell. Plasmid pLITMUS28 (New England Biolabs, Inc., Beverly, MA) is the backbone for the pNCT114 and pNCT224 gene targeting vector. The vector DNA comprises, at least (1) two DNA sequence homologous to the plastid genome (also referred as targeting sequence/fragment), (2) one or more promoter element, (3) transcription terminator elements, and (4) one or more selectable/drug resistance (non-lethal) marker gene.

Promoter element (PpsbA) DNA sequences were PCR amplified from genomic DNA extracted from 7 days old tobacco seedlings using the CTAB method as described by Murray and Thompson (*Nucleic Acids Res.*, [16] 8:4321-4325 (1980)). The primers used for amplification are listed (PpsbA forward primer: AACTGCAGGAATAGATCTACATACACCTTGG (SEQ ID NO:64), PpsbA reverse primer: CCGCTCGAGCTTAATTAAGGTAA

(cauliflower mosaic virus); and (3) right and left border T DNA repeats of Agrobacterium. Such a cassette could be introduced into plants either by a biolistic process or by Agrobacterium mediated gene transfer ([Horsch, et al., *Nature* 227:1229-1231 (1985))] (Horsch, et al., *Science* 227:1229-1231 (1985)). The cassette is based on pBI121 gene transfer vector (Jefferson, et al., *EMBO J.*, 6:3901-3907 (1987)). The design of the final cassette is illustrated in Figure 22.

In the biolistic process, the transforming DNA is coated on the surface of fine gold particles and introduced into the plant cell by a particle accelerator gun (PDS1000/He gun, Biorad, Richmond, CA). For Agrobacterium mediated gene transfer the transforming DNA cassette is introduced into the bacteria. The Agrobacterium harboring the cassette is allowed to be in contact with a disk or tissue section from tobacco or other suitable plant leaves. This facilitates the transfer of the DNA cassette to the plant nuclei. In either of the above approaches, the DNA finally gets integrated into the plant nuclei. The putative transformed cells are used for marker gene (drug) selection. The plants regenerated in presence of the selected drugs are strong transgenic candidates. After the plants are mature, the cell extracts will be taken and mixed with SDS loading dye with 1 mM DTT, boiled at 95°C for 5 min and loaded on a 10-20% Tris-glycine-SDS gradient gel. The separated proteins will be blotted on an Immobilin-P membrane and probed with an anti-ALS or EPSPS antibody.